Ground Beetle (Coleoptera: Carabidae) Assemblages in a Transgenic Corn–Soybean Cropping System

B. WADE FRENCH,[1, 2] LAURENCE D. CHANDLER,[3] MICHAEL M. ELLSBURY,[1] BILLY W. FULLER,[4] AND MARK WEST[5]

Environ. Entomol. 33(3): 554–563 (2004)

ABSTRACT Ground beetles often prey on crop pests, and their relative abundance and assemblages vary among cropping systems and pest management practices. We used pitfall traps arranged in transects to study ground beetle assemblages in a large field-scale Bt corn–soybean cropping system for 3 yr. The transgenic corn expressed the Cry1Ab protein targeting lepidopteran pests. Three of the 57 ground beetle species collected accounted for 81% of all individuals captured. Six other species accounted for an additional 14% of all beetles captured. Ground beetles were captured equally in cornfields and soybean fields. They also were captured most frequently at field edges, but many were captured within field centers. Canonical correspondence analysis was used to arrange ground beetles along environmental gradients. Years 2001 and 2002 were the primary variables separating assemblages of ground beetles along the first canonical axis. The second canonical axis further separated the 2000 assemblage of ground beetles. With the effects of year and field removed, ground beetles were classified with respect to crop association and distance into the fields along axes 1 and 2 of a partial canonical correspondence analysis. Based on this analysis, ground beetles occupying the Bt cornfields were separated from those occupying soybean fields along the first canonical axis. The second canonical axis separated beetles occupying the field borders from field interiors. Ground beetles ordinating near the center of the axes may represent habitat generalists, and because of their high relative abundances, continuous seasonal activity, predatory nature, and ability to occupy field centers, they could assist in the biological control of agricultural pests.

KEY WORDS Cry1Ab, nontarget, predators, crop rotation, canonical correspondence analysis

MOST GROUND BEETLES ARE generalist predators that search for prey at the soil surface of agricultural fields and adjacent habitats (Thiele 1977, Allen 1979, Luff 1987, Lövei and Sunderland 1996). Ground beetles prey on agricultural pests and are important in controlling pest populations in many agroecosystems (Sunderland and Vickerman 1980, Scheller 1984, Floate et al. 1990, Winder 1990, Ekbom et al. 1992, Holopainen and Helenius 1992, Sunderland et al. 1995). The effectiveness of ground beetles in controlling pests varies among cropping systems and cultural practices. There are many examples of ground beetle abundance and diversity being affected by cropping systems and pest management tactics (Rivard 1966, French et al. 1998, Gurr et al. 1998, Landis et al. 2000, Purvis and Fadl 2002). In the northern Great Plains of the United States, ground beetle diversity and species abundances were influenced more by crop rotations than by tillage practices (Weiss et al. 1990, Ellsbury et al. 1998). Also, to be effective in suppressing pest populations, ground beetles must be able to inhabit field interiors (Wissinger 1997, Landis et al. 2000).

Since 1996, corn (*Zea mays* L.) expressing the Cry1Ab protein has been available to producers. This protein is derived from the common soil bacterium, *Bacillus thuringiensis* (Berliner; Bt), and targets lepidopteran pests of corn such as the European corn borer (*Ostrinia nubilalis* Hübner). Producers in South Dakota have readily adopted this new corn into their typical cropping rotation. So far, corn expressing the Cry1Ab protein has shown little ecological effect on nontarget organisms including carabids (Lozzia 1999, Cannon 2000, Hunter 2000, Wraight et al. 2000, Stanley-Horn et al. 2001, Wold et al. 2001, Dale et al. 2002). However, long-term, large-scale field studies need to be conducted to determine community level changes of carabid beetles because of the deployment of the Cry1Ab protein (Cannon 2000, Hunter 2000, Dale et al. 2002).

As generalist predators, ground beetles can play an important role in keeping primary and secondary pest populations below economic thresholds (Potts and Vickerman 1974, Landis et al. 2000). Our objectives were (1) to ascertain the general species composition

[1] Northern Grain Insects Research Laboratory, USDA–ARS, 2923 Medary Ave., Brookings, SD 57006.

[2] E-mail: wfrench@ngirl.ars.usda.gov.

[3] Red River Valley Agricultural Research Center, USDA–ARS, P.O. Box 5677, Fargo, ND 58105.

[4] Department of Plant Science, South Dakota State University, Brookings, SD 57006.

[5] Northern Plains Area, USDA–ARS, 2150 Centre Ave., Suite 300, Ft. Collins, CO 80526.

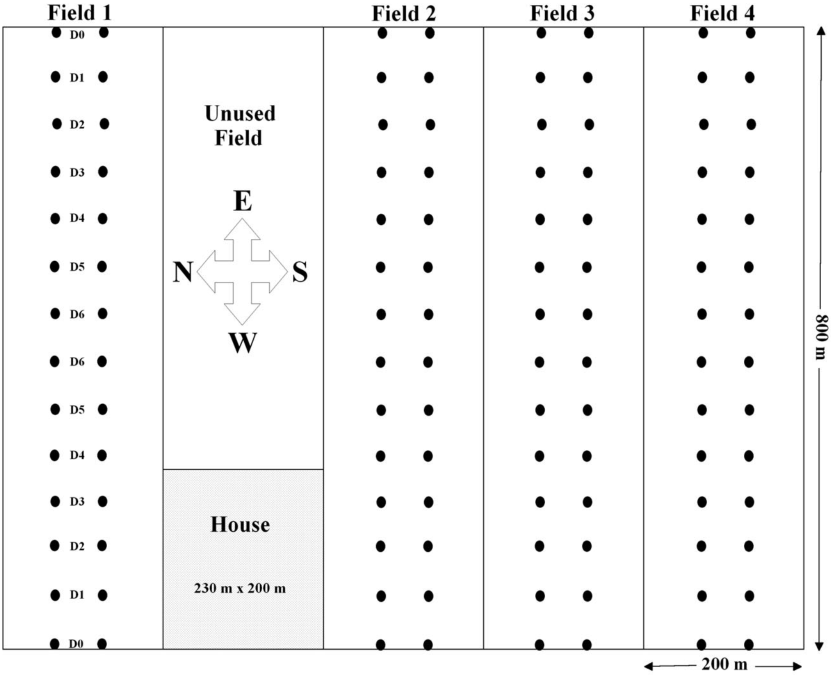

Fig. 1. Arrangement of fields and pitfall traps. Two east-to-west transects ≈60–80 m from the south (A transects) and north (B transects) boundaries were established along each of the four fields. Traps were placed at ≈60-m intervals from the borders. The borders represented an abrupt change in vegetation from crop to grass. Distance from border: D0 = 0 m, D1 = 60 m, D2 = 120 m, D3 = 180 m, D4 = 240 m, D5 = 300 m, and D6 = 360 m.

of ground beetle assemblages in southeastern South Dakota, (2) determine differences in species composition of ground beetles between crops in a Bt corn–soybean (*Glycine max* Merrill) cropping system, (3) describe the temporal structure of ground beetle assemblages in a Bt corn–soybean cropping system, and (4) describe the spatial structure of ground beetle assemblages in a Bt corn–soybean cropping system.

Materials and Methods

Study Area. This study was conducted in 2000, 2001, and 2002 in Brookings County, SD, on four fields of rotated corn and soybean in Aurora Township (west one-half of section 25, T110N, R49W). Two of the fields were started in corn and the remaining two in soybean. The fields ranged in size from 16.1 to 16.5 ha (≈200 by 800 m) and have been in a corn soybean rotation for several years before the study. On the north and south side of fields 1, 2, and 3, and on the north side of field 4, a single row (≈6.5 m wide) of Ponderosa pine trees, *Pinus ponderosa* Lawson, served as a windbreak. A 200 by 800-m field in corn–soybean rotation was on the north side of field 1, and a multiple row windbreak (≈30 by 800 m) was on the south side of field 4. A multiple row windbreak (≈200 by 230 m) near the house (Fig. 1) bordered a portion of the south side of field 1 and the north side of field 2. A grassy fence-row bordered the fields on the east, and a grassy roadside ditch bordered the fields on the west. The soil type at the study site is Brandt silty clay loam (taxonomic class: fine-silty, mixed, superactive, frigid Calcic Hapludolls; see http://soils.usda.gov). In all 3 yr, the corn planted expressed the Cry1Ab protein. Cornfields were planted each year between 19 April and 3 May. Soybean fields were planted each year between 15 and 30 May, and in 2002, glyphosate resistant soybeans were used. Each year, pre-emergence herbicides were applied to cornfields and soybean fields (Table 1). In 2002, the herbicides nicosulfuron + rimsulfuron, mesotrione, and atrazine were combined and applied to cornfields as a tank mixture. Fertilizer was also applied each year to cornfields but not to soybean fields (Table 1).

Pitfall Traps. We established two east-to-west transects ≈60–80 m from the south (A transects) and north (B transects) boundaries along each of the four fields. Fourteen pitfall traps were placed ≈60 m apart in each transect to capture ground beetles (Fig. 1). Trap design followed that of Morrill (1975) and consisted of a 455-ml Solo cup (Concept Communications, Burr Ridge, IL) with a 145-mm i.d., a Solo Cozy Cup funnel, and an inner 148-ml Solo cup partially filled with propylene glycol as a preservative. Pitfall traps were set in July of each year. We opened traps for 48 h each week through September (August in 2000). This sampling period covered the peak activity and most of the activity period for ground beetles in this area (Kirk 1971. Ground beetles were identified to species following the nomenclature of Bousquet and

Table 1. Type and amounts of fertilizer (kg/ha) and herbicides (liter/ha) applied to the crops each year

Year	Corn		Soybean	
	Fertilizer	Herbicide	Fertilizer	Herbicide
2000	168 N, 27 K, 0 P	Doubleplay 5.8	None	Raptor 0.4
2001	112 N, 34 K, 0 P	Doubleplay 5.6	None	Raptor 0.4
2002	163 N, 12 K, 19 P	Steadfast 0.06 Callisto 0.11 Atrazine 0.6	None	FirstRate 0.06

Doubleplay, Eradicane (EPTC + safener) + Surpass (acetochlor); Raptor, imazamox; FirstRate, cloransulam; Steadfast, nicosulfuron + rimsulfuron; Callisto, mesotrione.

Larochelle (1993). Voucher specimens are housed at the Northern Grain Insects Research Laboratory, USDA–ARS, Brookings, SD.

We used pitfall traps for the study because they are easy to install, effective for capturing Carabidae, and work continuously (Halsall and Wratten 1988). However, numbers generated from pitfall trap catches alone do not provide estimates of absolute density; rather, they provide estimates of activity densities, which is a function of a species population size, activity, and catchability (Greenslade 1964). However, activity density or "relative abundance" may be more important than absolute density in relation to biological control of pests, because active predators may be more likely to encounter prey than sedentary predators (Lenski 1982, Luff 1990). In addition, sampling continuously over a period of weeks or months with pitfall traps provides better data for estimating relative abundance of species, and abundances of particular species within a habitat for comparison of abundance among years or seasons in that habitat (Baars 1979). However, one must be cautious about interpreting differences in relative abundances among habitats, because species differ in catchability depending on trap type and habitat (Luff 1975, Halsall and Wratten 1988, Morrill et al. 1990, Spence and Niemelä 1994).

Data Analysis. We used PROC MIXED (SAS Institute 1988) to test for differences in relative abundance for each of nine predominant ground beetle species as well as for all nine combined, giving a total of 10 separate analyses. Our variables included crop, distance, year, field, rotation, and transect. Rotation indicated the history of the fields during our 3-yr study period (e.g., corn–soybean–corn or soybean–corn–soybean). Transects were classified as either A or B. We focused the analysis on differences in numbers captured by crop and distance into the fields. Distance was included as a categorical variable with levels D0, D1, D2, D3, D4, D5, and D6. The levels D0–D6 refer to the approximate distances into the fields (Fig. 1), with D0 representing border traps, D1 = 60 m, D2 = 120 m, D3 = 180 m, D4 = 240 m, D5 = 300 m, and D6 = 360 m. In our model, random effects included year, field, rotation, and transect. Species relative abundance data were transformed to square roots before analysis. Significant differences among crops and distances were based on type 3 tests of fixed effects. Because our interest was to compare relative abundance in border areas of the fields to their interiors, we tested each distance D1–D7 against D0 using Dunnett's procedure, controlling for the experiment-wise type 1 errors (SAS Institute 1988). Moreover, we controlled for the type 1 errors among all 10 analyses by making a Bonferroni adjustment. If ≤50 individuals of a particular species were captured, they were considered rare. No statistical tests were performed on the rare species, except that they were included in total trap catches and the canonical correspondence analyses.

The computer program CANOCO (ter Braak and Šmilauer 1998) was used to perform canonical correspondence analysis on species relative abundance data. Canonical correspondence analysis is an ordination technique that relates species relative abundances to environmental variables and is a robust method for analyzing data from pitfall traps (Palmer 1993). The environmental variables in a canonical correspondence analysis may include measured variables such as vegetation cover and temperature or they may include "dummy" variables such as seasons and habitats that represent temporal and spatial gradients (ter Braak 1986, 1995, Palmer 1993, ter Braak and Šmilauer 1998). Dummy variables, coded as 1 for presence or 0 for absence, were used in this analysis. We included the following 16 environmental variables: 2000, 2001, 2000, field 1, field 2, field 3, field 4, corn, soybean, D0, D1, D2, D3, D4, D5, and D6 in the initial analysis. We used the variables years and fields in the analysis to account for variability in species relative abundances. We used a partial canonical correspondence analysis to focus on the effect of the distance into the field and crop type on species relative abundance by using years and fields as co-variables, thereby accounting for their effects before conducting the analysis. All relative abundance data were transformed to square roots. We used Monte Carlo randomization tests to examine the significance of community patterns resulting from the canonical correspondence analyses (ter Braak and Šmilauer 1998).

Results

Species Data. Over all 3 yr, 24,750 ground beetles were captured, representing 57 species (Table 2). The beetles captured ranged in size from ≈2 mm for *Elaphropus anceps* (LeConte) and *Dyschirius globulosus* (Say) to ≈25 mm for *Calosoma calidum* (F.), *Harpalus caliginosus* (F.), and *Scarites subterraneus* (F.). Of the 57 species collected, three [*Cyclotrachelus alternans*

Table 2. Number and percentage of beetles captured for each species and abbreviations of species depicted in biplots of canonical correspondence analyses

Abbreviations	Species	No.	Percentage	Abbreviations	Species	No.	Percentage
Acp	*Acupalpus pauperculus* Dejean	3	<0.1	Cip	*Cicindela punctulata* Olivier	942	3.8
Agc	*Agonum cupripenne* (Say)	12	<0.1	Cir	*C. repanda* Dejean	1	<0.1
Agp	*A. placidum* (Say)	91	0.4	Clb	*Clivina bipustulata* (F.)	30	0.1
Ama	*Amara angustata* (Say)	1	<0.1	Cli	*C. impressefrons* LeConte	5	<0.1
Amc	*A. carinata* (LeConte)	91	0.4	Cya	*Cyclotrachelus alternans* (Casey)	8,949	36.2
Ame	*A. exarata* Dejean	4	<0.1	Cyp	*Cymindis pilosus* Say	2	<0.1
Ami	*A. impuncticollis* (Say)	1	<0.1	Dip	*Discoderus parallelus* (Haldeman)	3	<0.1
Aml	*A. latior* (Kirby)	2	<0.1	Dyg	*Dyschirius globulosus* (Say)	2	<0.1
Ali	*A. littoralis* Mannerheim	1	<0.1	Ela	*Elaphropus anceps* (LeConte)	96	0.4
Amo	*A. obesa* (Say)	33	0.1	Gaj	*Galerita janus* (F.)	2	<0.1
Anh	*Anisodactylus harrisii* LeConte	1	<0.1	Hac	*Harpalus caliginosus* (F.)	60	0.2
Anr	*A. rusticus* (Say)	36	0.1	Hac	*H. erraticus* Say	103	0.4
Ans	*A. sanctaecrucis* (F.)	5	<0.1	Haf	*H. faunus* Say	3	<0.1
Ban	*Badister notatus* Haldeman	1	<0.1	Hah	*H. herbivagus* Say	55	0.2
Bem	*Bembidion mimus* Hayward	3	<0.1	Hao	*H. opacipennis* (Haldeman)	1	<0.1
Beq	*B. quadrimaculatum* Say	891	3.6	Hap	*H. pensylvanicus* (DeGeer)	6,583	26.6
Ber	*B. rapidum* (LeConte)	22	<0.1	Hav	*H. ventralis* LeConte	10	<0.1
Brj	*Brachinus janthinipennis* (Dejean)	1	<0.1	Min	*Microlestes nigrinus* (Mannerheim)	159	0.6
Bro	*B. ovipennis* LeConte	244	1.0	Poc	*Poecilus chalcites* (Say)	425	1.7
Brq	*B. quadripennis* Dejean	2	<0.1	Pol	*P. lucublandus* (Say)	937	3.8
Cag	*Calathus gregarius* (Say)	99	0.4	Por	*Polyderis rufotestacea* (Hayward)	2	<0.1
Cac	*Calosoma calidum* (F.)	186	0.8	Ptc	*Pterostichus coracinus* (Newman)	1	<0.1
Cao	*C. obsoletum* Say	1	<0.1	Ptf	*P. femoralis* (Kirby)	11	<0.1
Cas	*Carabus serratus* Say	1	<0.1	Ptm	*P. melanarius* (Illiger)	3	<0.1
Che	*Chlaenius emarginatus* Say	1	<0.1	Ptp	*P. permundus* (Say)	4,450	18.0
Chp	*C. platyderus* Chaudoir	53	0.2	Scs	*Scarites subterraneus* F.	101	0.4
Chs	*C. sericeus* (Forster)	9	<0.1	Stc	*Stenolophus comma* (F.)	1	<0.1
Cht	*C. tomentosus* (Say)	6	<0.1	Syi	*Synuchus impuctatus* (Say)	1	<0.1
Ctr	*C. tricolor* Dejean	1	<0.1				

(Casey), *Harpalus pensylvanicus* (DeGeer), and *Pterostichus permundus* (Say)] accounted for 81% of all individuals captured (Table 2), and we consider these to be the dominant species. Six other species, [*Bembidion quadrimaculatum* Say, *Brachinus ovipennis* LeConte, *Calosoma calidum* (F.), *Cicindela punctulata* Olivier, *Poecilus chalcites* (Say), and *P. lucublandus* (Say)], accounted for an additional 14% of all individuals captured (Table 2), which we considered to be abundant species. If we collected a total of 50–175 individuals of a species, they were considered common. Rare species accounted for 38 of the 57 captured, and 15 species were captured only once (Table 2).

We found no significant interactions between crop and distance for the total number of beetles captured, each of the dominant species, or the six abundant species captured. Across all species, although more beetles were captured in the cornfields than in the soybean fields, the difference was not significant (Table 3). The three dominant species, *C. alternans, H. pensylvanicus,* and *P. permundus,* also were captured more often in the cornfields, but the differences were not significant (Table 3). There were no significant differences in numbers captured in cornfields and soybean fields for the other species (*B. quadrimaculatum, C. punctulata, P. chalcites,* and *P. lucublandus*) except for *C. calidum,* which was captured predominantly in soybean fields.

Over all species, we found significant differences in the number of beetles captured with respect to distance into the fields of Bt corn and soybean from the field edges ($F = 14.46$, df = 6,300, $P < 0.0001$; Table 4). The greatest numbers of ground beetles were captured at the field borders. For each distance from 60 to 360 m into the fields, the numbers captured differed significantly from the numbers captured in the border. Similarly, there also were significant differences in numbers captured for each of the three dominant species, *C. alternans* ($F = 7.10$, df = 6,300, $P < 0.0001$), *H. pensylvanicus* ($F = 3.84$, df = 6,300, $P = 0.0011$), and *P. permundus* ($F = 6.63$, df = 6,300, $P < 0.0001$), because they each were captured most often near the field borders (Table 4). There were, however, substantial numbers of all three species captured at each distance into the fields. Of the six abundant species, only *C. punctulata* ($F = 5.06$, df = 6,300, $P < 0.0001$) and *P. chalcites* ($F = 3.24$, df = 6,300, $P = 0.0043$) showed significant differences in numbers captured with respect to distance from the borders. However, these species also were captured in substantial numbers at each distance into the fields. Indeed, a multiple comparison test for *P. chalcites* indicated no significant differences with respect to distance from the border (Table 4). There were no significant differences in numbers captured with respect to distance for *B. quadrimaculatum, B. ovipennis, C. calidum,* and *P. lucublandus.*

Multivariate Analysis. The eigenvalues of the canonical correspondence analysis measure the proportion of total variation in ground beetle relative abundance explained by each respective axis (ter Braak 1986, 1995, ter Braak and Šmilauer 1998). The eigenvalues, based on species relative abundances, for axes 1–4 were 0.101, 0.070, 0.048, and 0.024. Axis 1 accounted for 31.5% of the species–environment relationship, and together with axis 2, accounted for 53.3% of the species–environment relationship. Axes 1–4

Table 3. Least square mean ± SE relative abundances per trap (n = 168 for each crop over all 3 yr) for species of ground beetles captured during 2000–2002 in fields of soybean and Bt corn

Species	Crop		ANOVA		
	Corn	Soybean	F	df	P
All species	78.36 ± 37.05	68.90 ± 37.05	0.50	1,5.37	0.511
B. quadrimaculatum	2.72 ± 0.74	2.58 ± 0.74	0.04	1,5.22	0.854
B. ovipennis	0.58 ± 0.27	0.87 ± 0.27	1.10	1,8	0.325
C. calidum	0.13 ± 0.33	0.99 ± 0.33	16.88	1,5.61	<0.01
C. punctulata	2.80 ± 1.70	2.80 ± 1.70	0.21	1,6.46	0.664
C. alternans	30.47 ± 15.57	22.80 ± 15.57	2.62	1,5.1	0.165
H. pensylvanicus	20.40 ± 14.15	18.79 ± 14.15	2.66	1,5.31	0.160
P. chalcites	1.17 ± 0.66	1.39 ± 0.66	1.09	1,5.79	0.338
P. lucublandus	2.74 ± 0.88	2.84 ± 0.88	3.31	1,4.39	0.137
P. permundus	14.92 ± 6.58	11.57 ± 6.58	0.38	1,5.66	0.563

accounted for 76.0% of the total species–environment relationship. A biplot of the most important environmental variables and species scores illustrates that axis 1 represents an annual gradient (Fig. 2). The 3 dominant, 6 abundant, and 10 common species are depicted in bold. Species names and abbreviations are given in Table 1. Environmental variables are represented by arrows, and a relatively long arrow positioned close to an axis indicates a strong relationship with that axis (ter Braak 1986, Palmer 1993), such as years 2001 and 2002 with axis 1 (Fig. 2). Ground beetles positioned close to the arrows have a strong association with that variable, such as *C. calidum* (Cac) and year 2001, whereas ground beetles occurring near the origin of the axes represent perennial species that were captured in relatively similar numbers over all 3 yr [e.g., *C. alternans* (Cya), *C. gregarius* (Cag), *C. platyderus* (Chp), and *P. permundus* (Ptp)]. Beetle assemblages that predominate in 2001 had positive values on axis 1 and ordinated to the right of axis 2. Beetle assemblages that predominate in 2002 had negative values on axis 1 and ordinated to the left of axis 2. The ground beetle assemblage associated with 2000 had positive values on axis 2 and ordinated above axis 1. The observed patterns for ground beetles with environmental variables were significantly different from random (Monte Carlo test statistic = 3.25, P = 0.005) (ter Braak and Šmilauer 1998).

Partial canonical correspondence analysis was used to depict the effects of crop types and distance from field borders on patterns of species relative abundance. In this partial analysis, the effects on species composition of years and fields were factored out as covariables. The eigenvalues for axes 1–4 were 0.049, 0.039, 0.011, and 0.006. Again, these values measure the amount of variation in species scores explained by their respective axes, with axis 1 explaining more variation in species scores than axes 2 or 3. Of the variation in species composition remaining after factoring out the covariables, axis 1 accounted for 40.6% of the species–environment relationship, and together with axis 2, accounted for 73.5% of the species–environment relationship. A biplot of the environmental variables and species scores reveal that crop types is closely associated with axis 1 (Fig. 3). The second axis separated ground beetle species occupying field edges from those occupying field interiors. Ground beetles occurring near the origin of the axes may represent habitat generalists [e.g., *B. quadrimaculatum* (Beq), *B. ovipennis* (Bro), *C. punctulata* (Cip), *C. alternans* (Cya), and *E. anceps* (Ela)], whereas species occurring far from the origin represent crop or edge specialists [e.g., *M. nigrinus* (Min), and *H. erraticus* (Hae)]. Species associated with soybeans ordinated in the positive space of axis 1 and ordinated to the right of axis 2 [e.g., *C. calidum* (Cac)], whereas species

Table 4. Least square mean ± SE relative abundances per distance from field borders (n = 48 traps for each distance over all 3 yrs) for species of ground beetles captured during 2000–2002 in fields of soybean and Bt corn

Species	Approximate distance from field border (m)						
	0	60	120	180	240	300	360
All species	105.87 ± 36.00a	75.42 ± 36.00b	65.73 ± 36.00b	61.54 ± 36.00b	66.29 ± 36.00b	70.38 ± 36.00b	70.19 ± 36.00b
B. quad	3.06 ± 0.76a	2.33 ± 0.76a	2.40 ± 0.76a	2.88 ± 0.76a	2.54 ± 0.76a	2.81 ± 0.76a	2.54 ± 0.76a
B. ovipennis	0.98 ± 0.28a	0.60 ± 0.28a	0.54 ± 0.28a	0.50 ± 0.28a	0.75 ± 0.28a	0.75 ± 0.28a	0.96 ± 0.28a
C. calidum	0.44 ± 0.35a	0.46 ± 0.35a	0.63 ± 0.35a	0.60 ± 0.35a	0.38 ± 0.35a	0.77 ± 0.35a	0.60 ± 0.35a
C. punctulata	5.60 ± 1.51a	3.23 ± 1.51b	2.52 ± 1.51b	1.98 ± 1.51b	1.92 ± 1.51b	2.48 ± 1.51b	1.90 ± 1.51b
C. alternans	37.00 ± 15.26a	30.27 ± 15.26b	24.54 ± 15.26b	21.60 ± 15.26b	24.04 ± 15.26b	24.71 ± 15.26b	24.27 ± 15.26b
H. pen	25.15 ± 13.64a	19.08 ± 13.64b	16.67 ± 13.64b	17.23 ± 13.64b	18.31 ± 13.64b	19.56 ± 13.64b	21.15 ± 13.64b
P. chalcites	1.73 ± 0.70a	2.00 ± 0.70a	1.77 ± 0.70a	1.10 ± 0.70a	0.69 ± 0.70b	0.69 ± 0.70b	0.88 ± 0.70a
P. lucublandus	3.85 ± 0.95a	2.58 ± 0.95a	2.54 ± 0.95a	2.06 ± 0.95a	2.75 ± 0.95a	2.60 ± 0.95a	3.13 ± 0.95a
P. permundus	20.02 ± 6.36a	11.92 ± 6.36b	12.00 ± 6.36b	11.08 ± 6.36b	11.58 ± 6.36b	13.94 ± 6.36b	12.17 ± 6.36b

Mean comparisons were tested against D0 using t-tests with Dunnett adjustments for type 1 errors; experiment-wise error rate set at α = 0.05

B. quad, Bembidion quadrimaculatum; H. pen, Harpalus pensylvanicus.

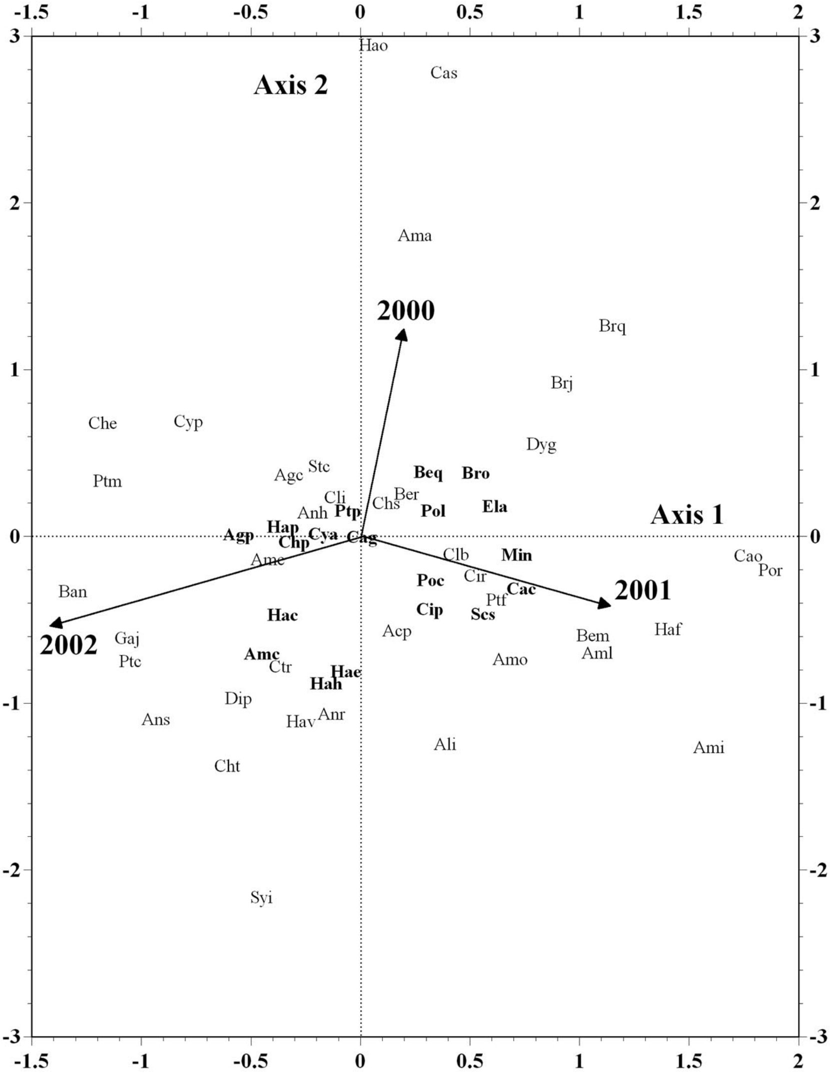

Fig. 2. Biplot of ground beetle relative abundances and most important environmental variables from the canonical correspondence analysis. The abbreviations of species names are plotted, and complete names are listed in Table 2. The 19 dominant, abundant, and common species are shown in bold. Arrows represent environmental variables.

associated with corn ordinated in the negative space of axis 1 and ordinated to the left of axis 2 [e.g., *H. pensylvanicus* (Hap)]. Species of ground beetles associated with field edges of both cornfields and soybean fields ordinated in the positive space of axis 2 [e.g., *C. gregarius* (Cag)]. Increasing distance from 60 to 360 m had little effect on ground beetle assemblages. The observed patterns for ground beetles with environmental variables were significantly different from random (Monte Carlo test statistic = 2.09, P = 0.005) (ter Braak and Šmilauer 1998).

Discussion

It is typical for a few species to dominate ground beetle assemblages in terms of relative abundance and to vary in numbers over space and time (Thiele 1977, Luff 2002). In this study, 3 species, *C. alternans, H. pensylvanicus,* and *P. permundus* (5.3%), of the 57 species collected accounted for 81% of all ground beetles captured. Six other species accounted for an additional 14%. Other studies also have found that a few species dominate the ground beetle fauna in agroecosystems (Kirk 1971, Barney and Pass 1986, Laub and Luna 1992, Tonhasca 1993, Cárcamo 1995, Ellsbury et al. 1998, French et al. 1998, French and Elliott 1999a, b). Although numbers captured differed from our study, the dominant and common species captured by Kirk (1971) and Ellsbury et al. (1998) in South Dakota were similar to those we captured. Both studies reported *C. alternans* as a dominant or most commonly found species. In contrast, Ellsbury et al.

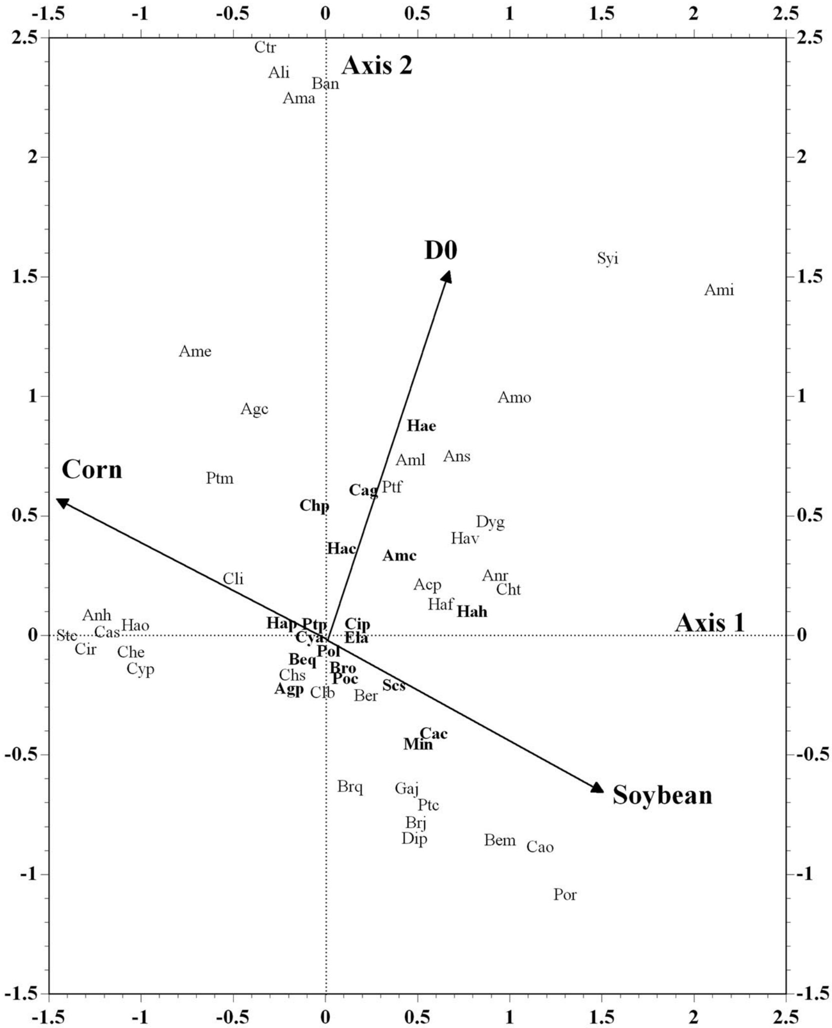

Fig. 3. Biplot of ground beetle relative abundances and environmental variables from a partial canonical correspondence analysis. The abbreviations of species names are plotted and complete names are listed in Table 2. The 19 dominant, abundant, and common species are shown in bold. Arrows represent environmental variables.

(1998) reported *P. permundus* as the fifth most abundant species, representing 3.1% of total catch, whereas Kirk (1971) reported rarely catching *P. permundus.* In our study, *P. permundus* accounted for 18% of total numbers captured. Kirk (1971) also reported *Pasimachus elongatus* LeConte as the most commonly found species; however, we did not catch a single individual of this species. It is unlikely that trap type affected the catchability of *P. elongatus* in our study because an identical trap type captured many *P. elongatus* in a study by French et al. 1998. *Harpalus pensylvanicus* is a wide-ranging species that has dominated catches in Ontario (Rivard 1964, 1966), Iowa (Esau and Peters 1975), Arkansas (Allen and Thompson 1977), North Carolina (Lesiewicz et al. 1983), North Dakota (Weiss et al. 1990), Georgia (Morrill 1992), Michigan (Clark et al. 1997), and Oklahoma (French et al. 1998, 2001, French and Elliott 1999a, b). The wide geographic range and dominance of *H. pensylvanicus* may be due in part to its omnivorous diet, which includes seeds and other insects (Kirk 1972, 1973, Best and Beegle 1977, Tyler and Ellis 1979, Shelton and Edwards 1983, Tooley and Brust 2002).

Across all species, ground beetles were captured equally in Bt cornfields and soybean fields. Similarly, considering the three dominant species, equal number of individuals of *C. alternans, H. pensylvanicus,* and *P. permundus* were captured in cornfields and soybean fields. For our study, this suggests that there were little differences between cornfields and soybean fields in factors such as relative humidity, soil moisture, temperature, and prey availability, affecting their distribution and relative abundances. As in our study, Ellsbury et al. (1998) found *C. alternans* and *H. pensylvanicus* to be dominant species in a corn/soybean cropping system. *H. pensylvanicus* also has been reported to be a dominant species in soybean fields (House and All 1981, Wiedenmann et al. 1992) and cornfields (Esau and Peters 1975, Best et al. 1981). In contrast, among the abundant species captured in our study, only *C. calidum* was captured in higher numbers in soybean fields than in cornfields. The factors determining habitat preference for *C. calidum* are unknown but may be due in part to differences in prey availability between cornfields and soybean fields. For example, *C. calidum* belongs to a genus commonly called "caterpillar hunters" because they tend to prey on lepidopteran larvae (Thiele 1977, Toft and Bilde 2002), and because the Cry3A1 toxin targets lepidopterans, subsequent reduction in lepidopteran larvae may cause *Calosoma* species to seek prey elsewhere, such as soybean fields or other habitats. Crop type had no impact on *B. quadrimaculatum, C. punctulata, P. chalcites,* and *P. lucublandus* because they were captured equally in the cornfields and soybean fields.

For ground beetles to be effective biological control agents against agricultural pests, they must be able to occupy the center of the arable fields (Wissinger 1997, Landis et al. 2000). The highest number of beetles captured occurred at the field borders; however, correspondent numbers of beetles were captured from 60 to 360 m into the fields. The three dominant species, *C. alternans, H. pensylvanicus,* and *P. permundus,* also were captured most frequently at the field borders; however, they too were captured in substantial numbers at field centers. In Iowa, both Esau and Peters (1975) and Best et al. (1981) found *H. pensylvanicus* predominantly in field edges, but they also captured many within cornfields. French and Elliott (1999a, b) captured *H. pensylvanicus* most often in natural habitats and their borders rather than in wheat fields. *B. quadrimaculatum, B. ovipennis, P. chalcites,* and *P. lucublandus* were captured equally throughout the fields, and because they were captured equally in the crop fields, probably represent habitat generalists. The distribution of *P. chalcites* varied only slightly from the borders into the field centers. In contrast to *H. pensylvanicus*, French and Elliott (1999a, b) captured *P. chalcites* most often in wheat field interiors rather than in natural habitats and field borders. French and Elliott regarded *P. chalcites* as a synanthropic species, meaning it shares a close association with human activities and has probably benefited from agriculture in general (Spence and Spence 1988). *C. calidum* also were captured equally throughout the fields; however, again they were more abundant in soybean fields. *C. punctulata* varied in numbers captured from the field borders to field centers. The distribution of *C. punctulata* was similar to the dominant species in that they were captured most often at the borders, yet substantial numbers were collected within field interiors. Although we measured distances into the fields from east to west, given the shape of the fields and location of transects, beetles may had only dispersed ≈60–80 m from the windbreak edges. This could have accounted for some of the similarities in beetle catches from 60 to 360 m into the fields.

Annual variation in the relative abundance and occurrence of ground beetle species can be expected in both temporary and permanent habitats (den Boer 1986, Luff 1990, 2002). French and Elliott (1999a) showed that annual captures were important separators of ground beetle assemblages, second only to season of occurrence. In our study, the canonical correspondence analysis showed that axes 1 and 2 separated beetle assemblages based on years captured. Axis 1 separated beetle assemblages based on years 2001 and 2002 and axis 2 on year 2000. We sampled beetles only through August during 2000 and probably missed some important autumn breeding species and trap catches such as *H. pensylvanicus.* This is also depicted on axis 1, where *H. pensylvanicus* (Hap) ordinated directly on this axis and toward year 2002. Note also in our study that the two other dominant species (Cya and Ptp) ordinated near the axes origins, indicating the evenness of their relative abundances in all 3 yr. Ground beetle species closely associated with particular years ordinated near the respective environmental arrow (e.g., Cac and 2001, Chp and 2002).

When we factored out the effects of years and fields as co-variables, cropping system was the primary environmental factor separating ground beetle assemblages, as indicated along axis 1. Other studies have shown cropping systems and management to be important environmental factors affecting ground beetle assemblages (Sanderson 1994, Purvis et al. 2001, Luff 2002). The field border was another important location for capturing ground beetles, as indicated by those species associated with axis 2. Also, based on partial canonical correspondence analysis, French and Elliott (1999a, b) and French et al. (2001) were able to classify ground beetles as habitat edge or interior species. For example, in their study, *H. pensylvanicus* ordinated near the axes centers of the partial canonical correspondence analyses, indicating a habitat generalist. In our study, *H. pensylvanicus* also ordinated near the axes center, indicating a habitat generalist, but with a slight tendency toward cornfields. Also in our study, ground beetle species closely associated with particular environmental variables (crop type and distance) ordinated near the respective arrow (e.g., Cac and Soybean, and Hae and D0/border). Note also in this study that the three dominant species of ground beetles (Cya, Pol, and Ptp) and many of the common species ordinated near the axes origins, indicating the evenness of their relative abundances over both crops and distances.

A small number of species accounted for a large portion of all ground beetles captured in all years and crops. *C. alternans, H. pensylvanicus,* and *P. permundus* were consistently captured in relatively high numbers, and along with several other species, were captured throughout the cornfields and soybean fields. Ground beetle assemblages were separated primarily by year and then by crop. The three dominant species and most of the abundant species ordinated near the axes origins, indicating stability over time and ability to occupy multiple habitats. Many species ordinated near field borders and probably represent edge species. It is not clear whether the abundant species spend their entire life cycle within the cornfields and soybean fields or overwinter in the field edges and disperse into the cornfields and soybean fields with time. However, their high relative abundances, continuous seasonal activity, predatory nature, and ability to occupy field centers make these carabid beetles good candidates for biological control of primary and secondary agricultural pests.

Acknowledgments

We thank V. and T. Hill for use of land. D. Beck, A. Beckler, J. Flaskey, D. Brink, S. DeBoer, D. Breitbarth, T. Telkamp, M. McPherson, B. Hoffman, S. Brende, S. Nedved, B. Johnston, and B. Johnston helped establish the traps and sort specimens. D. Beck identified the ground beetles to species. J. Burd and L. Hesler made valuable comments on the manuscript. Two anonymous reviewers kindly critiqued the manuscript. Mention of a proprietary product does not constitute an endorsement or a recommendation by the USDA for its use.

References Cited

Allen, R. T. 1979. The occurrence and importance of ground beetles in agricultural and surrounding habitats, pp. 485–505. *In* T. E. Erwin, G. E. Ball, D. R. Whitehead, and A. L. Halpern (eds.), Carabid beetles: their evolution, natural history, and classification. W. Junk, The Hague, The Netherlands.

Allen, R. T., and R. G. Thompson. 1977. Faunal composition and seasonal activity of Carabidae (Insecta: Coleoptera) in three different woodland communities in Arkansas. Ann. Entomol. Soc. Am. 70: 31–34.

Baars, M. A. 1979. Catches in pitfall traps in relation to mean densities of carabid beetles. Oecologia (Berl.). 41: 25–46.

Barney, R. J., and B. C. Pass. 1986. Ground beetle (Coleoptera: Carabidae) populations in Kentucky alfalfa and influence of tillage. J. Econ. Entomol. 79: 511–517.

Best, R. L., and C. C. Beegle. 1977. Food preferences of five species of carabids commonly found in Iowa cornfields. Environ. Entomol. 6: 9–12.

Best, R. L., C. C. Beegle, J. C. Owens, and M. Ortiz. 1981. Population density, dispersion, and dispersal estimates for *Scarites substriatus*, *Pterostichus chalcites*, and *Harpalus pensylvanicus* (Carabidae) in an Iowa cornfield. Environ. Entomol. 10: 847–856.

Bousquet, Y., and A. Larochelle. 1993. Catalogue of the Geadephaga (Coleoptera: Trachypachidae, Rhysodidae, Carabidae including Cicindelini) of America north of Mexico. Entomological Society of Canada, Ottawa, Ontario, Canada.

Cannon, R.J.C. 2000. *Bt* transgenic crops: risks and benefits. Int. Pest Manag. Rev. 5: 151–173.

Cárcamo, H. A. 1995. Effect of tillage on ground beetles (Coleoptera: Carabidae): a farm-scale study in central Alberta. Can. Entomol. 127: 631–639.

Clark, S. M., S. H. Gage, and J. R. Spence. 1997. Habitats and management associated with common ground beetles (Coleoptera: Carabidae) in a Michigan Agricultural Landscape. Environ. Entomol. 26: 519–527.

Dale, P. J., B. Clarke, and E.M.G. Fontes. 2002. Potential for the environmental impact of transgenic crops. Nat. Biotech. 20: 567–573.

den Boer, P. J. 1986. What can carabid beetles tell us about dynamics of populations, pp. 315–330. *In* P. J. den Boer, M. L. Luff, D. Mossakowski, and F. Weber (eds.), Carabid beetles, their adaptations and dynamics. Fisher, Stuttgart, Germany.

Ekbom, B. S., S. Wiktelius, and P. A. Chiverton. 1992. Can polyphagous predators control the bird cherry-oat aphid (*Rhopalosiphum padi*) in spring cereals? Entomol. Exp. Appl. 65: 215–223.

Ellsbury, M. M., J. E. Powell, F. Forcella, W. D. Woodson, S. A. Clay, and W. E. Riedell. 1998. Diversity and dominant species of ground beetle assemblages (Coleoptera: Carabidae) in crop rotation and chemical input systems for the northern Great Plains. Ann. Entomol. Soc. Am. 91: 619–625.

Esau, K. L., and D. C. Peters. 1975. Carabidae collected in pitfall traps in Iowa cornfields, fencerows, and prairies. Environ. Entomol. 4: 509–513.

Floate, K. D., J. F. Doane, and C. Gillott. 1990. Carabid predators of the wheat midge (Diptera: Cecidomyiidae) in Saskatchewan. Environ. Entomol. 19: 1503–1511.

French, B. W., and N. C. Elliott. 1999a. Temporal and spatial distribution of ground beetle assemblages in grasslands and adjacent wheat fields. Pedobiologia. 43: 73–84.

French, B. W., and N. C. Elliott. 1999b. Spatial and temporal distribution of ground beetle assemblages in riparian strips and adjacent wheat fields. Environ. Entomol. 28: 597–607.

French, B. W., N. C. Elliott, and R. C. Berberet. 1998. Reverting conservation reserve program lands to wheat and livestock production: effects on ground beetle (Coleoptera: Carabidae) assemblages. Environ. Entomol. 27: 1323–1335.

French, B. W., N. C. Elliott, R. C. Berberet, and J. D. Burd. 2001. Effects of riparian and grassland habitats on ground beetle (Coleoptera: Carabidae) assemblages in adjacent wheat fields. Environ. Entomol. 30: 225–234.

Greenslade, P.J.M. 1964. Pitfall trapping as a method for studying populations of Carabidae (Coleoptera). J. Anim. Ecol. 33: 301–310.

Gurr, G. M., H. F. van Emden, and S. D. Wratten. 1998. Habitat manipulation and natural enemy efficiency: implications for the control of pests, pp 155–183. *In* P. Barbosa (ed.), Conservation biological control. Academic, New York.

Halsall, N. B., and S. D. Wratten. 1988. The efficiency of pitfall trapping for polyphagous predatory Carabidae. Ecol. Entomol. 13: 293–299.

Holopainen, J. K., and J. Helenius. 1992. Gut contents of ground beetles (Col., Carabidae), and activity of these and other epigeal predators during an outbreak of *Rhopalosiphum padi* (Hom., Aphididae). Acta Agric. Scand. 42: 57–61.

House, G. J., and J. N. All. 1981. Carabid beetles in soybean agroecosystems. Environ. Entomol. 10: 194–196.

Hunter, M. D. 2000. Between hyperbole and hysteria: entomological issues and the deployment of transgenic plants. Agric. For. Entomol. 2: 77–84.

Kirk, V. M. 1971. Ground beetles in cropland in South Dakota. Ann. Entomol. Soc. Am. 64: 238–241.

Kirk, V. M. 1972. Seed-caching by larvae of two ground beetles, *Harpalus pensylvanicus* and *H. erraticus*. Ann. Entomol. Soc. Am. 65: 1426–1428.

Kirk, V. M. 1973. Biology of a ground beetle, *Harpalus pensylvanicus*. Ann. Entomol. Soc. Am. 66: 513–518.

Landis, D. A., S. D. Wratten, and G. M. Gurr. 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. Annu. Rev. Entomol. 45: 175–201.

Laub, C. A., and J. M. Luna. 1992. Winter cover crop suppression practices and natural enemies of armyworm (Lepidoptera: Noctuidae) in no-till corn. Environ. Entomol. 21: 41–49.

Lenski, R. W. 1982. Effects of forest cutting on two *Carabus* species: evidence for competition for food. Ecology. 63: 1211–1217.

Lesiewicz, D. S., J. W. Van Duyn, and J. R. Bradley Jr. 1983. Determinations on cornfield carabid populations in northeastern North Carolina. Environ. Entomol. 12: 1636–1640.

Lövei, G. L., and K. D. Sunderland 1996. Ecology and behavior of ground beetles (Coleoptera: Carabidae). Annu. Rev. Entomol. 41: 231–256.

Lozzia, G. C. 1999. Biodiversity and structure of ground beetle assemblages (Coleoptera: Carabidae) in Bt corn and its effects on non target insects. Boll. Zool. Agr. Bachic. 31: 37–58.

Luff, M. L. 1975. Some features influencing the efficiency of pitfall traps. Oecologia (Berl.). 19: 345–357.

Luff, M. L. 1987. Biology of polyphagous ground beetles in agriculture. Agric. Zool. Rev. 2: 237–278.

Luff, M. L. 1990. Spatial and temporal stability of carabid communities in a grass/arable mosaic, pp. 191–200. *In* N. E. Stork (ed.), The role of ground beetles in ecological and environmental studies. Intercept, Andover, UK.

Luff, M. L. 2002. Carabid assemblage organization and species composition, pp. 41–80. *In* J. M. Holland (ed.), The agroecology of carabid beetles. Intercept, Andover, UK.

Morrill, W. L. 1975. Plastic pitfall trap. Environ. Entomol. 4: 596.

Morrill, W. L. 1992. Ground beetles (Coleoptera: Carabidae) in Georgia grasslands. J. Agric. Entomol. 9: 179–188.

Morrill, W. L., D. G. Lester, and A. E. Wrona. 1990. Factors affecting efficacy of pitfall traps for beetles (Coleoptera: Carabidae and Tenebrionidae). J. Entomol. Sci. 25: 284–293.

Palmer, M. W. 1993. Putting things in even better order: the advantages of canonical correspondence analysis. Ecology. 74: 2215–2230.

Potts, G. R., and G. P. Vickerman. 1974. Studies on the cereal ecosystem. Adv. Ecol. Res. 8: 107–197.

Purvis, G., and A. Fadl. 2002. The influence of cropping rotations and soil cultivation practice on the population ecology of carabids (Coleoptera: Carabidae) in arable land. Pedobiologia. 46: 452–474.

Purvis, G., A. Fadl, and T. Bolger. 2001. A multivariate analysis of cropping effects on Irish ground beetle assemblages (Coleoptera: Carabidae) in mixed arable and grass farmland. Ann. Appl. Biol. 139: 351–360.

Rivard, I. 1964. Observations on the breeding periods of some ground beetles (Coleoptera: Carabidae) in eastern Ontario. Can. J. Zool. 42: 1081–1084.

Rivard, I. 1966. Ground beetles (Coleoptera: Carabidae) in relation to agricultural crops. Can. Entomol. 98: 189–195.

Sanderson, R. A. 1994. Carabidae and cereals: a multivariate approach, pp. 457–463. *In* K. Desender, M. Dufrêne, M. Loreau, M. L. Luff, and J. P. Maelfait (eds.), Carabid beetles: ecology and evolution. Kluwer, Dordrecht, Germany.

SAS Institute 1988. SAS/STAT user's guide, release 6.03. SAS Institute, Cary, NC.

Scheller, H. V. 1984. The role of ground beetles (Carabidae) as predators on early populations of cereal aphids in spring barley. Z. Angew. Entomol. 97: 451–463.

Shelton, M. D., and C. R. Edwards. 1983. Effects of weeds on the diversity and abundance of insects in soybeans. Environ. Entomol. 12: 296–283.

Spence, J. R., and D. H. Spence. 1988. Of ground beetles and men: introduced species and the synanthropic fauna of western Canada. Mem. Entomol. Soc. Can. 144: 151–168.

Spence, J. R., and J. K. Niemelä. 1994. Sampling carabid assemblages with pitfall traps: the madness and the method. Can. Entomol. 126: 881–894.

Stanley-Horn, D. E., G. P. Dively, R. L. Hellmich, H. R. Mattila, M. K. Sears, R. Rose, L.C.H. Jesse, J. E. Losey, J. J. Obrycki, and L. Lewis. 2001. Assessing the impact of Cry1Ab-expressing corn pollen on monarch butterfly larvae in field studies. Proc. Natl. Acad. Sci. U.S.A. 98: 11931–11936.

Sunderland, K. D., and G. P. Vickerman. 1980. Aphid feeding by some polyphagous predators in relation to aphid density in cereal fields. J. Appl. Ecol. 17: 389–396.

Sunderland, K. D., G. L. Lövei, and J. Fenlon. 1995. Diets and reproductive phenologies of the introduced ground beetles *Harpalus affinis* and *Clivina australasiae* (Coleoptera: Carabidae) in New Zealand. Aust. J. Zool. 43: 39–50.

ter Braak, C.J.F. 1986. Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. Ecology. 67: 1167–1169.

ter Braak, C.J.F. 1995. Ordination, pp. 91–169. *In* R.H.G. Jongman, C.J.F. ter Braak, and O.F.R. van Tongeren (eds.), Data analysis in community and landscape ecology. Cambridge University Press, Cambridge, UK.

ter Braak, C.J.F., and P. Šmilauer. 1998. CANOCO reference manual and user's guide to canoco for windows: software for canonical community ordination (version 4). Microcomputer Power, Ithaca, NY.

Thiele, H. U. 1977. Carabid beetles in their environments: a study on habitat selection by adaptations in physiology and behavior. Springer, New York.

Toft, S., and T. Bilde. 2002. Carabid diets and food value, pp. 81–110. *In* J. M. Holland (ed.), The agroecology of carabid beetles. Intercept, Andover, UK.

Tonhasca, A., Jr. 1993. Carabid beetle assemblage under diversified agroecosystems. Entomol. Exp. Appl. 68: 279–285.

Tooley, J., and G. E. Brust. 2002. Weed seed predation by carabid beetles, pp. 215–229. *In* J. M. Holland (ed.), The agroecology of carabid beetles. Intercept, Andover, UK.

Tyler, B.M.J., and C. R. Ellis. 1979. Ground beetles in three tillage plots in Ontario and observations on their importance as predators of the northern corn rootworm, *Diabrotica longicornis* (Coleoptera: Chysomelidae). Proc. Entomol. Soc. Ont. 110: 65–73.

Weiss, M. J., E. U. Balsbaugh, Jr., E. W. French, and B. K. Hoag. 1990. Influence of tillage management and cropping system on ground beetle (Coleoptera: Carabidae) fauna in the Northern Great Plains. Environ. Entomol. 19: 1388–1391.

Wiedenmann, R. N., P. L. Larrain, and R. J. O'Neil. 1992. Pitfall sampling of ground beetles (Coleoptera: Carabidae) in Indiana soybean fields. J. Kansas Entomol. Soc. 65: 279–291.

Winder, L. 1990. Predation of the cereal aphid *Sitobion avenae* by polyphagous predators on the ground. Ecol. Entomol. 15: 105–110.

Wissinger, S. A. 1997. Cyclic colonization in predictably ephemeral habitats: a template for biological control in annual crop systems. Biol. Control. 10: 4–15.

Wold, S. J., E. C. Burkness, W. D. Hutchison, and R. C. Venette. 2001. In-field monitoring of beneficial insect populations in transgenic corn expressing a *Bacillus thuringiensis* toxin. J. Entomol. Sci. 36: 177–187.

Wraight, C. L., A. R. Zangerl, M. J. Carroll, and M. R. Berebaum. 2000. Absence of toxicity of *Bacillus thuringiensis* pollen to black swallowtails under field conditions. Proc. Natl. Acad. Sci. U.S.A. 97: 7700–7703.

Received 16 July 2003; accepted 23 February 2004.